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Supplementary Materials for

Poly(GP) proteins are a useful pharmacodynamic marker for *C9ORF72*-associated amyotrophic lateral sclerosis

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Materials and Methods

Evaluating associations between CSF poly(GP) and behavior or cognitive impairment

c9ALS and c9ALS-FTD patients seen at the Mayo Clinic or the National Institutes of Health (NIH) were systematically screened for behavioral or cognitive function using a battery of validated neuropsychological tests that included assessment of attention, executive function, cognitive speed, memory, visual spatial skill, naming, verbal fluency, mood, and behavior (**table S8**). Assessments of behavioral or cognitive function were derived from an independent review of test results by two neuropsychologists (O.P. and B.K.R) at the Mayo Clinic, or by a neuropsychologist (E.W.) at the NIH (with the assistance of M.T.). Behavioral or cognitive status of study participants was normalized by reducing the neuropsychological assessments to a dichotomous scale (0=normal, 1=impaired) with respect to behavior or cognition. This approach acknowledges the limited sample size and our interest in evaluating at a basic level any associations between poly(GP) and neuropsychological status in c9ALS patients. Inter-rater discrepancies in ratings at the respective sites were reconciled between raters within sites without knowledge of poly(GP) data.

Preparation of peripheral blood mononuclear cells

Blood from a peripheral vein of *C9ORF72* repeat expansion carriers (N=36) and non-carriers (N=34) was collected in acid citrate dextrose (ACD) tubes using standard phlebotomy procedures at the Mayo Clinic, University of Miami, or Washington University School of Medicine. Tubes from the latter two institutes were shipped to the Mayo Clinic the day of collection. Samples were processed within 48 h of collection by gently transferring blood to a 15 ml tube containing 5 ml of Lymphoprep™ (Stemcell Technologies, cat. No. 07801), followed by centrifugation at room temperature for 30 min at 800 x g. The mononuclear cell phase was then transferred to a new 15 ml tube, washed with 10 ml of Hank's Balanced Salt Solution (HBSS, Thermo Fisher Scientific 14175079), and centrifuged at 200 x g for 10 min. The resulting cell pellet was resuspended in 2 ml of HBSS and carefully applied to 2 ml of Lymphoprep™ in a fresh tube. Following centrifugation for 10 min at 800 x g the mononuclear phase was washed once more before pelleting the cells by centrifugation at 1600 x g for 5 min, removing all HBSS, and storing the PBMCs at -80°C until protein was extracted from them. To extract protein, cell pellets were lysed in cold Co-IP Buffer (50 mM Tris-HCL, pH 7.4, 300 mM NaCl, 5mM EDTA, 1% Triton-X 100, 2% sodium dodecyl sulfate, 0.01% protease and phosphatase inhibitors), and sonicated on ice. Protein lysate was cleared by centrifugation at 16,100 x g for 20 min at 4°C. Supernatant was collected and protein concentrations were determined by a bicinchoninic acid (BCA) protein assay. Poly(GP) in PBMC lysates were measured by poly(GP) immunoassay (described below) blinded to the mutation and disease status of subjects.

Immunoassay analysis of poly(GP)

Poly(GP) in CSF were measured blinded to *C9ORF72* mutation and disease status using a previously described sandwich immunoassay that utilizes Meso Scale Discovery electrochemiluminescence detection technology, and an affinity purified rabbit polyclonal poly(GP) antibody (Rb9259) as both capture and detection antibody (*17*, *19*). Each CSF sample was tested in duplicate wells using 90 µl per well. Each assay plate contained the same control samples (post-mortem CSF from a *C9ORF72* mutation carrier and a non-carrier) to monitor inter-plate consistency. Serial dilutions of recombinant $(GP)_8$ in TBS were used to prepare the standard curve. Response values corresponding to the intensity of emitted light upon electrochemical stimulation of the assay plate using the Meso Scale Discovery QUICKPLEX SQ120 were acquired and background corrected using the response from the negative CSF control sample prior to interpolating poly(GP) concentrations (presented as ng/ml) using a five-parameter logistic equation.

Using the same assay, we measured poly(GP) in: PBMC lysates (60 ug/well), conditioned media (130 µl/well) or lysates (60 µg/well) from lymphoblastoid cell lines, conditioned media (130 µl/well) or lysates (35 µg/well) from iPSNs, CSF from $(G_4C_2)_2$- or $(G_4C_2)_{66}$-expressing mice (3 µl of CSF in 50 µl TBS per well), or mouse brain tissues (5 µg/well). All lysates were diluted to the required concentration using TBS. Concentrations of poly(GP) in lysates are presented as ng of poly(GP) per mg protein in homogenate (ng/mg). All samples were tested in duplicate wells.

Detection of poly(GP) in CSF using alternate immunoassays

To show that the detection of poly(GP) in CSF is not unique to one immunoassay, we assessed poly(GP) in 14 CSF samples from *C9ORF72* repeat expansion carriers using an Meso Scale Discovery-based assay that employed our rabbit polyclonal poly(GP) antibody (Rb9259, 0.5 µg/ml) as the capture antibody, and a chimeric (rabbit variable region and mouse IgG2a constant region) monoclonal anti-GP antibody (0.5 µg/ml, Biogen ABH596/597) as the detection antibody, coupled with SULFO-tag anti-mouse secondary antibody (0.25 µg/ml, Meso Scale Discovery).

We additionally tested the same 14 CSF samples on the Quanterix HD-1 Analyzer using a 2-step Simoa poly(GP) assay. In the first step, CSF, anti-GP antibody (Rb9259)-coated paramagnetic capture beads and biotinylated detector antibodies were combined. After washing, a conjugate of streptavidin-ß-galactosidase (SßG) was mixed with the capture beads to bind the biotinylated detector antibodies, thus enzyme labeling the captured poly(GP). Following a second wash, the capture beads were resuspended in a resorufin ß-Dgalactopyranoside (RGP) substrate solution and transferred to the Simoa array. Individual capture beads were sealed within microwells in the array, and fluorescent signal resulting from ß-galactosidase hydrolysis of the captured, labeled poly(GP) was measured. Data output was transcribed into Average Enzymes per Bead (AEB).

Associations between poly(GP) responses obtained using our traditional immunoassay to responses obtained using the second Meso Scale Discovery immunoassay or the Quanterix HD-1 Analyzer were evaluated using a Spearman's test of correlation.

Lymphoblastoid cell lines and c9ASO-1 treatment

Lymphoblastoid cell lines were grown in Iscove's Modified Dulbecco's Medium (IMDM) (Hyclone, SH30228.01) plus 10% FBS, 1% pen-strep and Amphotericin B (2.5 mg/ml). To examine poly(GP) in lymphoblastoid cell lysates and media, lymphoblastoid cell lines from individuals with or without a *C9ORF72* repeat expansion were seeded at 1.8×10^7 cells per T25 flask. Four days later, cells were spun down at 600 x g for 5 min, media was collected and frozen, and cells were washed once with phosphate buffered saline (PBS), prior to extracting protein using Co-IP buffer (as described above) for poly(GP) analysis by immunoassay. For c9ASO treatment, lymphoblastoid cell lines were seeded at 4.5×10^6 cells per T25 flask. A previously described ASO that targets the hexanucleotide G_4C_2 repeat sequence (*12*) was purchased from Integrated DNA Technologies. Specific ASO details are as follows: 1) Sequence: CCGGCCCCGGCCCCGGCCCC; 2) Modification: 5-10-5, 2'-O-methyl RNA (OME), phosphorothioate backbone; and 3) Function: RNase H activation. We denote this ASO as c9ASO-1. As a control ASO, we used an ASO (CCTTCCCTGAAGGTTCCTCC) generated by Ionis Pharmaceuticals. Lymphoblastoid cell lines were treated at a final concentration of 5 μM. Three and six days after seeding, cells were titrated and treated again with 2.5 μM control ASO or c9ASO-1. Ten days after seeding, cells were harvested for protein extraction using Co-IP buffer (as described above) for poly(GP) analysis by immunoassay, RNA extraction for quantitative real-time PCR (qRT-PCR, described below), or used for RNA fluorescence in situ hybridization (FISH) to visualize RNA foci (described below).

RNA extraction and quantitative real-time PCR for lymphoblastoid cell lines

Total RNA was extracted from lymphoblastoid cells using the Direct-zol™ RNA MiniPrep kit (Zymo Research) according to the manufacturer's instructions, combined with an in-column DNase I digestion step. Then, 2 μg of total RNA was used for reverse transcription to synthesize cDNA using random primers and the High Capacity cDNA Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. To quantify mRNA of *C9ORF72* variants, qRT-PCR was conducted in triplicate for all samples using the SYBR green assay (Life Technologies) on an ABI Prism 7900HT Fast Real-Time PCR System (Applied Biosystems). The primers used were as follows: *C9ORF72* variant 1: 5'-CCACGTAAAAGATGACGCTTGATA-3' and 5'-TGGGCAAAGAGTCGACATCA-3'; *C9ORF72* variant 2: 5'-CGGTGGCGAGTGGATATCTC-3' and 5'-TGGGCAAAGAGTCGACATCA-3'; *C9ORF72* variant 3: 5'-GCAAGAGCAGGTGTGGGTTT-3' and 5'-TGGGCAAAGAGTCGACATCA-3'; *RPLP0*: 5′-TCTACAACCCTGAAGTGCTTGAT-3′ and 5′-CAATCTGCAGACAGACACTGG-3′. Relative mRNA expression of examined genes was normalized to *RPLP0* values, the endogenous transcript control.

RNA fluorescence in situ hybridization (FISH) analysis and quantification of RNA foci in lymphoblastoid cell lines

Lymphoblastoid cell lines (2.0×10^5 cells) treated with control ASO or c9ASO-1 were titrated and then loaded onto poly-L lysine-coated coverslips (Wescor, SS-118) using CytoSpin at 1, 000 rpm for 5 min. After drying, cells were fixed in 4% PFA, and then washed with DEPC-treated PBS. Next, fixed cells were permeabilized with 0.2% Triton/DEPC-PBS, and then dehydrated by 70%, 70% and 100% ethanol washes (1 min per wash). Cells were then incubated for 30 min at 66°C in hybridization solution. During this time, LNA probes to detect sense (/5TYE563/CCCCGGCCCGGCCCC) or antisense (/5TYE563/GGGGCCGGGGCCGGGG) *C9ORF72* repeat expansions were heated at 80°C for 75 sec and then diluted to 40 nM in hybridization solution. Cells were incubated with the diluted LNA probes at 66°C for 24 h. Cells were then washed once with 2 × SSC/0.1% Tween-20 for 5 min at room temperature, and 3 times with 0.1 × SSC for 5 min per wash at 60°C, followed by counterstaining with Hoechst 33258 (1 μg/ml, Thermo Fisher Scientific). Next, cells were dehydrated through 70%, 70% and 100% ethanol washes (1 min per wash), and the coverslips were mounted on slides. Images were obtained on a Zeiss LSM700 laser scanning confocal microscope. To quantify foci-bearing cells, ~300 total cells per coverslip were analyzed to determine the average percentage of foci-positive cells for each treatment.

iPSN differentiation and c9ASO-2 treatment

c9ALS and control patient fibroblasts were reprogrammed into iPSCs using the four vector retrovirus method, and differentiated into a mixed neuronal population (90% Tuj1-positive, 30-40% HB9-positive) via embryoid body formation, as previously described (*12*). The size of the G_4C_2 repeat expansion in iPSC lines was determined by Southern blotting as described elsewhere (*4*). On day 28, neural progenitor cells were plated on Matrigel (Corning) at equal densities in T25 flasks or 6-well plates. On day 32, neurons were treated with Ara-C (Sigma) for 48–72 h to remove glial progenitor cells. Modified 2'-*O*-methoxyethyl (MOE)/DNA ASOs were generated by Ionis Pharmaceuticals, as previously reported (*12*, *22*). At day 45 of differentiation, *C9ORF72* intron 1 ASO (TACAGGCTGCGGTTGTTTCC), termed c9ASO-2, or control ASO (CCTTCCCTGAAGGTTCCTCC), was added directly to culture media at concentrations of 0.3 μM, 3 μM, or 30 μM without the use of a transfection reagent. ASOs were replaced with media changes every five days.

iPSN sample collection for poly(GP) concentration determination

Media was collected at day 0 (prior to initiation of ASO treatment) and every five days thereafter for the measurement of poly(GP) by immunoassay. Following collection, media was centrifuged to remove any dead cells and protease inhibitor (Roche) was added prior to freezing media at -80°C until further use. Prior to measuring poly(GP) in media, Triton X-100 was added to a final concentration of 1%, and media was concentrated five-fold using an Amicon Ultra 10 kDa cutoff filter (Millipore). On day 20, iPSN were rinsed with PBS and lysed in ice-cold RIPA buffer

(Sigma) with protease inhibitor. Following centrifugation, the supernatant was collected and protein concentrations were determined by BCA assay.

iPSN RNA extraction and quantitative real-time PCR

Total RNA was isolated 20 days after ASO treatment initiation using the miRNeasy Kit with on-column DNase treatment (Qiagen). RNA quantification was performed via Nanodrop (Thermo). qRT-PCR was performed using Taqman probes and Express One-Step reagents (Thermo), with primers and probe sequences as follows (*22*); all human *C9ORF72* variants: forward – TGTGACAGTTGGAATGCAGTGA, reverse – GCCACTTAAAGCAATCTCTGTCTTG, probe – TCGACTCTTTGCCCACCGCCA. Repeat-containing human *C9ORF72* variants: forward – GGGTCTAGCAAGAGCAGGTG, reverse – GTCTTGGCAACAGCTGGAGAT, probe – TGATGTCGACTCTTTGCCCACCGC.

Mouse studies

All procedures using mice described in this study were performed in accordance with the National Institutes of Health Guide for Care and Use of Experimental Animals and approved by the Mayo Clinic Institutional Animal Care and Use Committee.

Generation of AAV9-$(G_4C_2)_2$ and AAV9-$(G_4C_2)_{66}$ plasmids

Plasmids containing the $(G_4C_2)_2$ and $(G_4C_2)_{66}$ repeat expansions were generated as previously described (*20*). In brief, the $(G_4C_2)_2$ or $(G_4C_2)_{66}$ repeats with 119 base pairs of the 5' flanking region of the *C9ORF72* gene and 100 base pairs of 3' flanking region, as well as a triple tag in which each c9RAN protein expresses a specific tag at the end of its translated product (GR is FLAG, GA is HA, and GP is MycHis) were inserted into the HindIII and XhoI restriction sites of the adeno-associated virus (AAV) expression vector pAM/CBA-pl-WPRE-BGH, which contains inverted repeats of serotype 2. AAV-$(G_4C_2)_2$ and AAV-$(G_4C_2)_{66}$ particles were packaged into serotype 9 type capsid and purified using standard methods. In brief, AAV vectors containing $(G_4C_2)_2$ and $(G_4C_2)_{66}$ repeats were generated by plasmid transfection with helper plasmids into HEK293T cells. Sixty-four hours after transfection, the cells were harvested and lysed in the presence of 0.5 % sodium deoxycholate and 50 Units/ml Benzonase (Sigma-Aldrich) by freeze thawing, and the virus was isolated using a discontinuous iodixanol gradient. The genomic titer of each virus was determined by quantitative PCR. AAV stocks were diluted with PBS prior to injection.

Neonatal viral injections

Intracerebroventricular (ICV) injections of AAV into neonatal mouse brains at postnatal day 0 (P0) were performed as previously described (*20*). In brief, P0 mice were cryoanesthetized on ice for approximately 3 min or until pups exhibited no movement. For the injection, a 32-gauge needle (Hamilton Company) attached to a 10 µl syringe (Hamilton Company) was positioned

approximately two-fifths the distance between the lambda suture and each of the eyes of C57BL/6J pups and the needle was inserted at a 30-degree angle from the surface of the head and held at a depth of approximately two millimeters. Two microliters (1E10 genomes/µl) of AAV2/9-$(G_4C_2)_2$ or AAV2/9-$(G_4C_2)_{66}$ solution was manually injected into each cerebral ventricle. After injections, pups were placed on a heat pad until they completely recovered from anesthesia and then were placed back into their home cages.

Antisense oligonucleotide injections in mouse central nervous system, and cerebrospinal fluid and tissue collection

c9ASO-1 or PBS was injected into the central nervous system of 4–4.5 month-old C57BL/6J mice by means of stereotactic ICV injection, as previously described with some minor modifications (*22*). Specifically, 10 µl of c9ASO-1 solution, which corresponds to a total of 500 µg ASOs, or 10 µl of PBS, as a control, were administered into the right ventricle by using the subsequent coordinates: 0 mm anterior and 1.0 mm lateral to the right from bregma and 1.9-2.0 mm deep as measured from the brain surface. Mice were aged to 6.5 months of age, and then anesthetized with 90-120/10 mg/kg of ketamine/xylazine through intraperitoneal administration. The depth of anesthesia was evaluated by toe and tail pinch. The skin and fur covering the top of head and neck of mice were removed to reveal the subcutaneous tissue and muscles underneath. Muscles overlaying the magna cisterna were cut and moved to the sides. Once the dura mater overlaying the magna cisterna was visible, the area was cleansed with cotton swabs soaked in sterile PBS and dried to remove any blood around the area to prevent contamination of CSF. Excessive bleeding around the area was ceased by a cauterizer pen. A 26 5/8 gauge needle was used to gently penetrate into the magna cisterna through the dura mater, and CSF was collected with a gel loading tip into protein low binding tubes (Eppendorf). CSF was immediately frozen on dry ice. Volume of CSF collected per mouse ranged from 8–15 µl. Mice were subsequently euthanized by cervical dislocation and tissues were collected. Efficiency of RNA knock-down was determined from hemibrains, excluding cerebellum, by qRT-PCR as described below. At least 9 mice were used per condition. PBS treated AAV2/9-$(G_4C_2)_2$ mice (n=12) or AAV2/9-$(G_4C_2)_{66}$ mice (n=11), and c9ASO-1 treated AAV2/9-$(G_4C_2)_2$ mice (n=10) and AAV2/9-$(G_4C_2)_{66}$ (n=9) mice were compared.

Preparation of brain lysates and RNA extraction

Frozen half brains, excluding the cerebellum, of PBS-treated AAV2/9-$(G_4C_2)_2$ mice (n=12) or AAV2/9-$(G_4C_2)_{66}$ mice (n=10), and c9ASO-1-treated AAV2/9-$(G_4C_2)_2$ mice (n=11) and AAV2/9-$(G_4C_2)_{66}$ (n=9) mice were sonicated in ice cold Tris-EDTA buffer (Sigma) with 2x protease and phosphatase inhibitors at 1:5 w/v ratio with brief pulses at 25% power until tissue was completely dissolved. The resulting homogenate was used to prepare brain lysates and RNA. One-hundred-fifty microliters of each brain homogenate was mixed with 2x lysis buffer (50 mM Tris pH 7.4, 250 mM NaCl, 2% Triton X-100, 4% SDS, and protease and phosphatase inhibitors). Brain lysates were sonicated at 1 s on/off intervals for 15 s to ensure complete lysis and then spun at 13,000 rpm for 20 min at 4°C and supernatants were collected. Protein concentrations were measured by BCA assay (Pierce Biotechnology), and lysates were stored at -

20°C until immunoassay analyses of poly(GP) (described above) and poly(GA) (described below). For RNA extraction, brain homogenates were combined in 1 to 3 volumes of Trizol LS Reagent (Life Technologies) and stored at -80°C. Total RNA was subsequently extracted from brain homogenates using the Direct-zol RNA MiniPrep kit (Zymo Research) according to the manufacturer's instructions.

Immunoassay analysis of poly(GA)

Poly(GA) in mouse brain lysates were measured using a Meso Scale Discovery sandwich immunoassay that utilizes a mouse monoclonal anti-GA antibody (4 µg/ml, 2P36E2, Biogen) as capture antibody, and a rabbit polyclonal anti-GA antibody (4 µg/ml, Rb2930, Biogen), along with a SULFO-tag anti-rabbit secondary antibody (2 µg/ml), to detect captured poly(GA). By assaying synthetic peptides of $(PA)_8$, $(PR)_8$, $(GR)_8$, $(GP)_8$ and $(GA)_8$ using 1 µg/well in duplicate wells, this assay was found to specifically detect poly(GA) and no other c9RAN protein (**fig. S5**). Each mouse brain lysate was tested in duplicate wells using 25 µg of protein per well. Serial dilutions of recombinant $(GA)_8$ in TBS were used to prepare the standard curve. Response values corresponding to the intensity of emitted light upon electrochemical stimulation of the assay plate using the Meso Scale Discovery QUICKPLEX SQ120 were acquired and poly(GA) concentrations (presented as ng/mg protein) were interpolated from the standard curve using a five-parameter logistic equation.

Quantitative real-time PCR in mouse tissue

Quantification of mRNA of exogenous G_4C_2-containing transcripts was performed using primers upstream of the repeat. Endogenous *C9orf72* and *Gapdh* mRNA were also quantified. In brief, cDNA of all samples was obtained following reverse transcription of 1 µg of the extracted RNA with random primers and the High Capacity cDNA Transcription Kit (Applied Biosciences) by following manufacturer's instructions. For the quantification of mRNA in mouse brain, qRT-PCR was performed in triplicate for all samples using the SYBR green assay (Life Technologies) on an ABI Prism 7900HT Fast Real-Time PCR System (Applied Biosystems). The sequences of the primers used for this study are as follows: G_4C_2-containing transcripts: 5'-TACAGCTCCTGGGCAACG-3'and 5'-CTTGTTCACCCTCAGCGAGT-3'; *C9orf72*: 5'-GGCAGCAGTGCAGAGAAAGT-3' and 5'-TCCTCTCTGCTGGTGTCAGA-3'; *Gapdh*: 5'-CATGGCCTTCCGTGTTCCTA-3' and 5'-CCTGCTTCACCACCTTCTTGAT-3'. Relative mRNA expression of all examined genes were normalized to the endogenous transcript control, *Gapdh*.

RNA fluorescence in situ hybridization (FISH) on mouse brain tissue

RNA FISH was performed as previously described (*20*). In brief, paraformaldehyde fixed-paraffin embedded (FFPE) brain sections underwent a series of xylene washes for deparaffinization and rehydrated through a series of ethanol solutions. Brain sections were placed in ice cold 2% acetone/1x DEPC-PBS for 5 min for permeabilization, washed twice with

DEPC-water, and dehydrated with a series of ethanol solutions. Sections were first incubated with pre-hybridization buffer [50% formamide (Midsci), 10% dextran sulfate (Millipore), 2x saline-sodium citrate buffer (SSC), 50 mM sodium phosphate buffer, pH 7.0] for 30 min at 66°C and hybridized with 40 nM of locked nucleic acid probe (LNA) [TYE563-(CCCCGGCCCCGGCCCC; Exiqon 500150, batch: 612968, (*22*)] for 24 h at 66°C in a humidified, dark chamber. Sections were subsequently washed once in 2x SSC/0.1% Tween-20 for 5 min, and washed twice in pre-warmed 0.2x SSC at 66°C for 10 min in the dark. Slides were mounted with Vectashield mounting media with DAPI (Vector Laboratories). Representative images of RNA foci were taken with a Zeiss Axio Imager Z1 fluorescent microscope (Carl Zeiss MicroImaging) under 63x magnification. Quantitative analysis of foci-bearing cells in the motor cortex of PBS-treated AAV2/9-$(G_4C_2)_{66}$ mice (n=6, 300 cells/mouse) and c9ASO-1-treated AAV2/9-$(G_4C_2)_{66}$ mice (n=6, 300 cells/ mouse) was undertaken. Images of the motor cortex were taken at 40x magnification, with multiple images taken per field to ensure all RNA foci-positive cells were visible. The number of cells positive for one or more RNA foci was counted from a total of 300 cells per mouse, and the percentage of foci-positive cells was calculated.

Immunohistochemical staining of mouse brain sections

Immunohistochemical staining of mouse brain sections was performed as previously described (*20*). In brief, FFPE brain sections (5 µm; sagittal sections) were deparaffinized in xylene washes and rehydrated with a series of ethanol solutions. Slides were placed in boiling distilled water for 30 min for antigen retrieval. Then, sections were immunostained with rabbit polyclonal antibodies against poly(GA) (Rb9880, 1:50,000), poly(GP) (Rb5823, 1:10,000) or poly(GR) (Rb7810, 1:2500) using the DAKO Autostainer (Universal Staining System) with the DAKO + HRP system. Slides were counterstained with hematoxylin, dehydrated through a series of ethanol and xylene washes, and mounted with Cytoseal mounting media (Thermo Fisher Scientific, Inc). Slides were scanned using ScanScope® AT2 (Leica Biosystems) at a 40x magnification. Representative images of poly(GA)-, poly(GP)-, and poly(GR)-positive cells were obtained from ImageScope® software (v12.1; Leica Biosystems). Quantitative analyses of the number of cells positive for poly(GA), poly(GP), or poly(GR) in the motor cortex were performed manually on scanned images from ImageScope® software on brain sections of PBS-treated AAV2/9-$(G_4C_2)_{66}$ (n=6) and c9ASO-1-treated AAV2/9-$(G_4C_2)_{66}$ (n=5).

Supplementary Figures

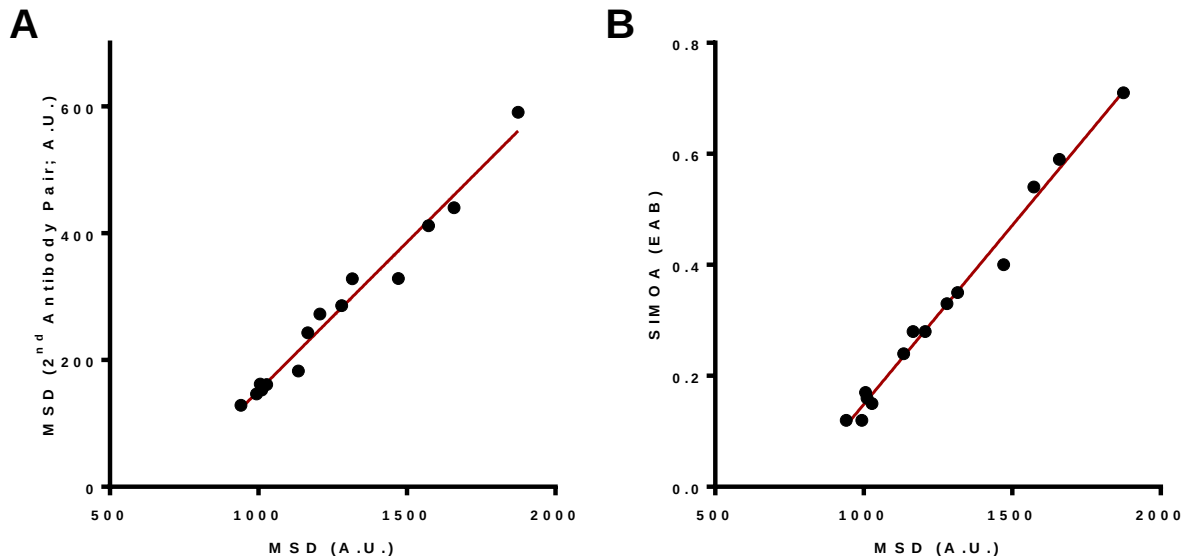

Fig. S1. Validation of poly(GP) detection in CSF. Our traditional poly(GP) Meso Scale Discovery-based immunoassay, which utilizes the rabbit poly(GP) antibody, Rb9259, as both the detection and capture antibody, has been employed to detect poly(GP) in various preclinical models of c9ALS, as well as cerebrospinal fluid (CSF) from c9ALS patients. (**A**) To establish that poly(GP) can be detected in CSF using more than one immunoassay, we compared measures of poly(GP) in 14 CSF samples from *C9ORF72* repeat expansion carriers obtained using our traditional assay to measures obtained using an Meso Scale Discovery-based assay that utilizes a different antibody pair (capture antibody: rabbit polyclonal anti-GP antibody, Rb9259; detection antibody: rabbit/mouse chimeric monoclonal anti-GP antibody). (**B**) A similar comparison was made with poly(GP) measures obtained using a prototype assay established on the Simoa HD-1 Analyzer, which is based on digital single molecule measurements. Like our traditional assay, the Simoa assay used the rabbit polyclonal anti-GP antibody, Rb9259, for both capture and detection of the antigen. Shown in panels **A** and **B** are associations between responses corresponding to the intensity of emitted light upon electrochemical stimulation of the assay plate (arbitrary units, AU) from the Meso Scale Discovery assays, and the average number of enzymes per bead (AEB) from the Simoa assay, both of which reflect the number of poly(GP) immunocomplexes formed.

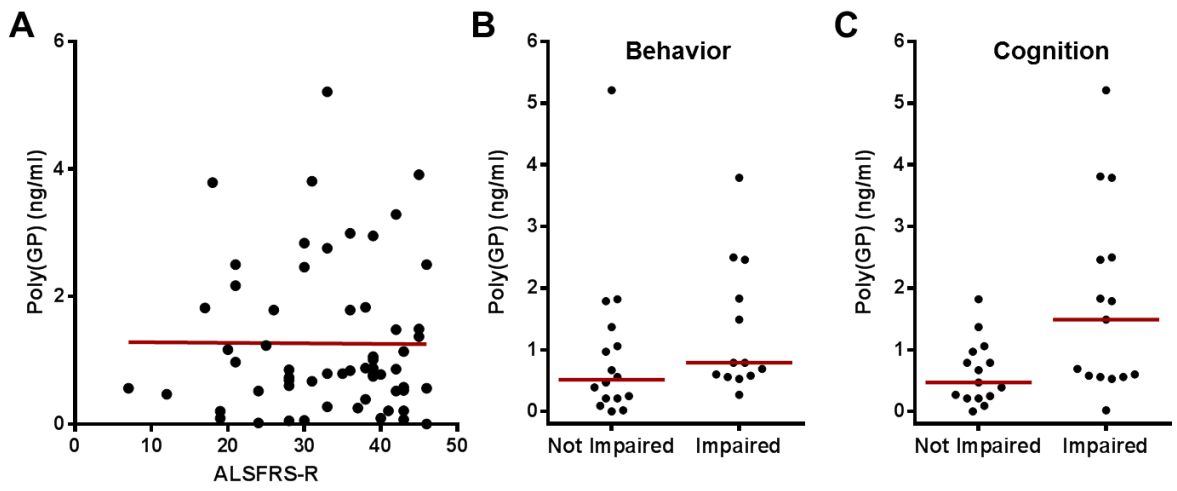

Fig. S2. Relationships between CSF poly(GP) and ALSFRS-R score, behavioral impairment, and cognitive impairment. (**A**) Association between CSF poly(GP) and ALSFRS-R score of c9ALS and c9ALS-FTD patients (N=60). (**B, C**) Patients with c9ALS or c9ALS-FTD seen at the Mayo Clinic or NIH, and for whom neuropsychological testing for behavioral and cognitive function was carried-out, were rated as normal or impaired with regards to behavior or cognition. Shown in panels **B** and **C** are CSF poly(GP) concentrations in patients with or without behavioral (N=29) or cognitive (N=30) impairment. The sample median in a given group is denoted by a solid horizontal line. See also related data in tables S7–S10.

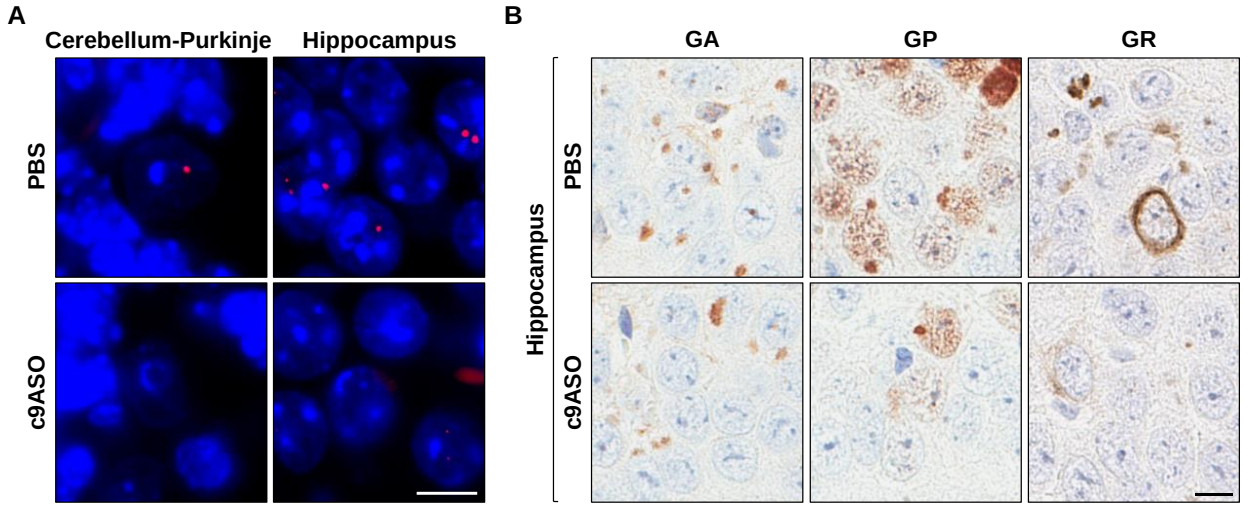

Fig. S3. Decreases in RNA foci and c9RAN protein pathology are observed in $(G_4C_2)_{66}$ mice treated with c9ASO-1. At 4–4.5 months of age, $(G_4C_2)_{66}$ mice received a single ICV bolus injection of PBS or c9ASO-1. (**A**) Treatment with c9ASO-1 for 8 weeks significantly decreased the number cells with RNA foci in the cerebellum and hippocampus. (**B**) Likewise, fewer cells with inclusions immunopositive for poly(GA), poly(GP), or poly(GR) were observed in the hippocampus of c9ASO-1-treated $(G_4C_2)_{66}$ mice. Scale bar, 10 µm. See also related data in Fig. 5, and fig. S4.

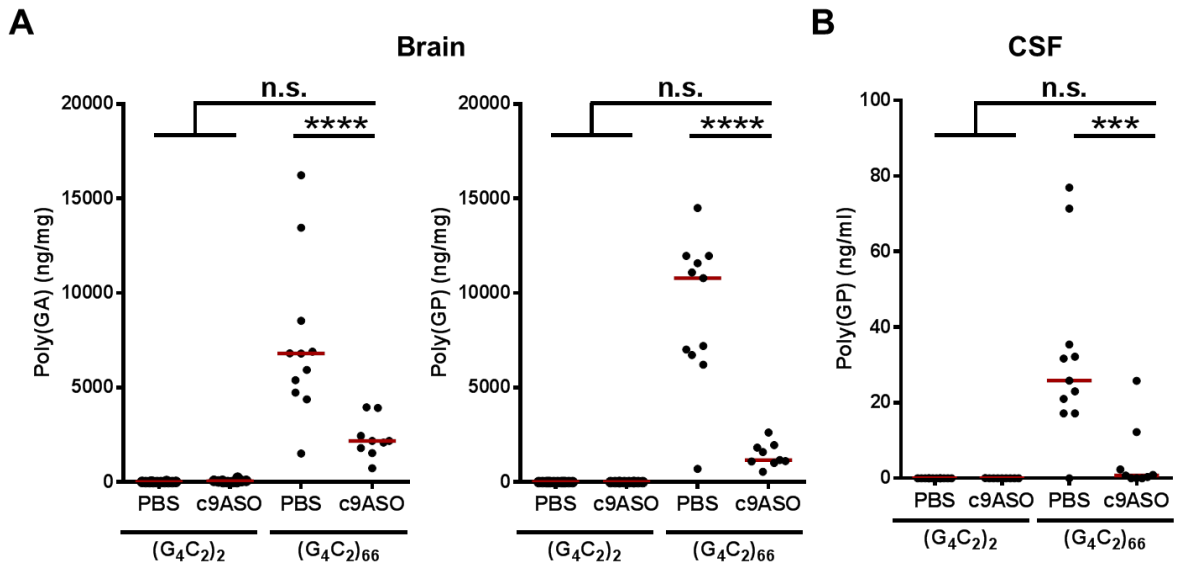

Fig. S4. Decreases in brain and CSF poly(GP) are observed in $(G_4C_2)_{66}$ mice treated with c9ASO-1. At 4–4.5 months of age, $(G_4C_2)_2$ mice and $(G_4C_2)_{66}$ mice were treated with a single ICV bolus injection of PBS or c9ASO-1. Eight weeks later, CSF was collected from mice prior to harvesting tissues for biochemical and immunohistochemical analyses. (**A**) Concentrations of poly(GA) or poly(GP) were measured in hemibrain homogenates by immunoassay. (**B**) Concentrations of poly(GP) were also measured in mouse CSF. ***P<0.001, ****P<0.0001, as assessed by one-way ANOVA followed by Tukey's multiple comparisons. Note the significant decrease in poly(GP) and poly(GA) in brain homogenates and/or CSF from c9ASO-1-treated $(G_4C_2)_{66}$ mice compared to $(G_4C_2)_{66}$ mice treated with PBS only. Red horizontal line indicates the median. See also related data in Fig. 5, and fig. S3.

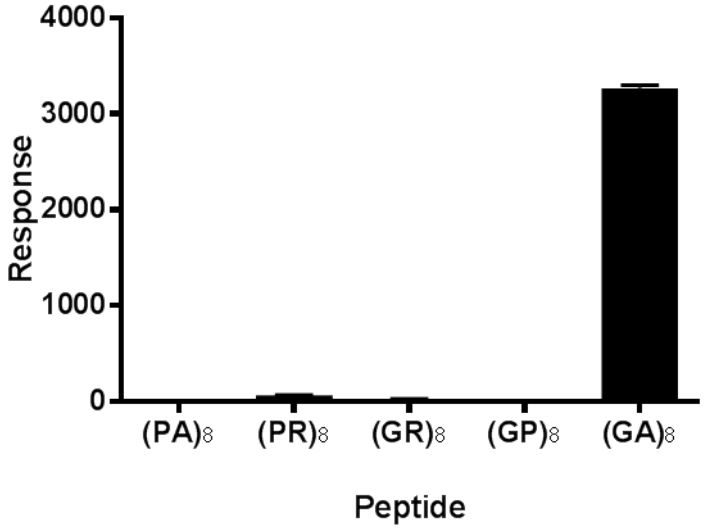

Fig. S5. Validation of poly(GA) Meso Scale Discovery sandwich immunoassay. To validate specificity of the poly(GA) assay, synthetic peptides representing each possible c9RAN protein translated from sense or antisense transcripts of the expanded *C9ORF72* repeat were diluted in Tris-buffered saline (TBS) and assayed (1 µg per well in duplicate wells). Response values correspond to intensity of emitted light upon electrochemical stimulation of the assay plate using the Meso Scale Discovery QUICKPLEX SQ120, from which the background response in wells containing only TBS was subtracted. Data presented as mean + standard deviation of responses in duplicate wells.

Table S1. Distribution of the 254 subjects among the sites that provided CSF.

	Non-*C9ORF72* repeat expansion carriers			*C9ORF72* repeat expansion carriers		
	Healthy controls (N=48)	ALS or ALS-FTD (N=57)	Other diseases (N=15)	Asymptomatic (N=27)	ALS or ALS-FTD (N=83)	Other diseases (N=24)
Site						
A	21 (43.8%)	10 (17.5%)	10 (66.7%)	1 (3.7%)	10 (12.0%)	5 (20.8%)
B	3 (6.3%)	0 (0.0%)	0 (0.0%)	6 (22.2%)	16 (19.3%)	3 (12.5%)
C	2 (4.2%)	19 (33.3%)	0 (0.0%)	5 (18.5%)	14 (16.9%)	2 (8.3%)
D	21 (43.8%)	4 (7.0%)	0 (0.0%)	11 (40.7%)	2 (2.4%)	0 (0.0%)
E	0 (0.0%)	17 (29.8%)	5 (33.3%)	0 (0.0%)	17 (20.5%)	1 (4.2%)
F	0 (0.0%)	7 (12.3%)	0 (0.0%)	0 (0.0%)	10 (12.0%)	0 (0.0%)
G	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	9 (10.8%)	13 (54.2%)
H	1 (2.1%)	0 (0.0%)	0 (0.0%)	4 (14.8%)	5 (6.0%)	0 (0.0%)

A: Emory University School of Medicine. B: National Institutes of Health. C: Mayo Clinic Jacksonville. D: University of Miami. E: IRCCS Istituto Auxologico Italiano - Università degli Studi di Milano and Ospedale Maggiore Crema. F: Massachusetts General Hospital, University of Massachusetts Medical School, Barrow Neurological Institute, University of Pittsburgh Medical Center. G: University of Pennsylvania. H: Washington University School of Medicine.

Table S2. Poly(GP) in CSF from *C9ORF72* repeat expansion carriers and noncarriers.

Non-*C9ORF72* repeat expansion carriers						*C9ORF72* repeat expansion carriers					
Healthy controls (N=48)		ALS (N=57)		Other diseases (N=15)		Asymptomatic (N=27)		ALS or ALS-FTD (N=83)		Other diseases (N=24)	
Patient number	Poly(G) (ng/ml)	Patient number	Poly(GP) (ng/ml)	Patient number	Poly(GP) (ng/ml)	Patient number	Poly(GP) (ng/ml)	Patient number	Poly(GP) (ng/ml)	Patient number	Poly(GP) (ng/ml)
1	0.00	49	0.00	106	0.00	121	1.36	148	0.09	231	1.10
2	0.00	50	0.00	107	0.00	122	1.58	149	0.84	232	0.37
3	0.00	51	0.00	108	0.00	123	0.13	150	0.78	233	0.03
4	0.00	52	0.00	109	0.00	124	0.95	151	2.95	234	0.00
5	0.00	53	0.00	110	0.00	125	0.29	152	0.88	235	1.28
6	0.00	54	0.00	111	0.00	126	0.05	153	2.99	236	0.26
7	0.00	55	0.00	112	0.00	127	0.15	154	3.91	237	0.85
8	0.00	56	0.06	113	0.00	128	0.08	155	0.52	238	2.04
9	0.01	57	0.00	114	0.00	129	0.30	156	1.01	239	1.51
10	0.00	58	0.00	115	0.00	130	0.00	157	1.48	240	0.35
11	0.00	59	0.00	116	0.00	131	0.19	158	1.84	241	0.30
12	0.00	60	0.00	117	0.00	132	0.94	159	0.86	242	0.38
13	0.00	61	0.00	118	0.00	133	0.51	160	0.56	243	0.82
14	0.00	62	0.00	119	0.00	134	1.46	161	1.82	244	2.25
15	0.00	63	0.00	120	0.00	135	0.68	162	0.97	245	1.14
16	0.00	64	0.00			136	3.98	163	0.21	246	0.20
17	0.00	65	0.00			137	0.36	164	0.39	247	2.62
18	0.00	66	0.00			138	0.31	165	0.27	248	2.14
19	0.00	67	0.00			139	0.81	166	0.67	249	1.35
20	0.00	68	0.00			140	1.60	167	3.81	250	2.38
21	0.00	69	0.00			141	0.05	168	2.46	251	5.25
22	0.00	70	0.00			142	1.09	169	0.25	252	1.77
23	0.00	71	0.00			143	0.28	170	1.79	253	1.09
24	0.00	72	0.00			144	0.55	171	0.69	254	0.48
25	0.00	73	0.00			145	0.64	172	3.79		
26	0.00	74	0.00			146	0.49	173	5.21		
27	0.00	75	0.00			147	0.43	174	0.00		
28	0.00	76	0.00					175	0.20		
29	0.00	77	0.00					176	0.05		
30	0.00	78	0.00					177	0.73		
31	0.00	79	0.01					178	1.00		
32	0.00	80	0.00					179	1.17		
33	0.00	81	0.00					180	0.88		
34	0.04	82	0.00					181	1.79		
35	0.00	83	0.00					182	2.17		
36	0.00	84	0.00					183	1.02		
37	0.00	85	0.00					184	0.38		
38	0.00	86	0.00					185	2.84		
39	0.00	87	0.00					186	0.06		
40	0.00	88	0.00					187	2.76		
41	0.00	89	0.00					188	1.23		
42	0.00	90	0.00					189	0.58		
43	0.00	91	0.00					190	0.53		
44	0.00	92	0.00					191	2.50		
45	0.00	93	0.00					192	1.83		
46	0.00	94	0.00					193	1.06		
47	0.00	95	0.00					194	0.56		
48	0.01	96	0.00					195	0.00		

	97	0.00			196	0.79
	98	0.00			197	1.37
	99	0.00			198	0.21
	100	0.00			199	0.79
	101	0.00			200	0.47
	102	0.00			201	0.09
	103	0.00			202	1.49
	104	0.00			203	0.02
	105	0.00			204	0.60
					205	0.07
					206	0.85
					207	1.60
					208	1.66
					209	0.52
					210	0.25
					211	0.03
					212	3.29
					213	0.78
					214	0.00
					215	1.52
					216	1.14
					217	0.68
					218	0.52
					219	0.75
					220	0.72
					221	2.50
					222	5.24
					223	0.83
					224	0.00
					225	0.74
					226	1.48
					227	0.47
					228	0.93
					229	0.96
					230	0.85

Table S3. Associations of poly(GP) with *C9ORF72* repeat expansion and disease status.

Variable	N	Poly(GP)	P value	
			Unadjusted	Adjusted
C9ORF72 repeat expansion status			<0.0001	<0.0001[a]
Negative	120	0.0 (0.0, 0.0, 0.0, 0.1)		
Positive	134	0.8 (0.0, 0.4, 1.5, 5.3)		
C9ORF72 repeat expansion carriers			0.047	0.42[b]
Asymptomatic	27	0.5 (0.0, 0.2, 1.0, 4.0)		
ALS or ALS-FTD	83	0.8 (0.0, 0.5, 1.6, 5.2)		

The sample median (minimum, 25th percentile, 75th percentile, maximum) is given. [a]van Elteren stratified Wilcoxon rank sum test, where 12-strata were created according to age, gender, and disease group. [b]Linear regression model adjusted for age at CSF collection and gender. See also Tables S4 and S5 for characteristics of patients involved in these comparisons.

Table S4. Characteristics according to *C9ORF72* repeat expansion carrier status.

Variable	Non-*C9ORF72* repeat expansion carriers (N=120)	*C9ORF72* repeat expansion carriers (N=134)	P value
Age at CSF collection (Years)	57.6 (23.2, 47.0, 64.3, 85.2)	57.7 (28.0, 50.9, 63.6, 77.1)	0.54
Gender (Male)	65 (54.2%)	71 (53.0%)	0.90
Disease group			0.002
Asymptomatic	48 (40.0%)	27 (20.1%)	
ALS or ALS-FTD	57 (47.5%)	83 (61.9%)	
Other	15 (12.5%)	24 (17.9%)	

The sample median (minimum, 25th percentile, 75th percentile, maximum) is given for continuous variables. P values result from Fisher's exact test or a Wilcoxon rank sum test.

Table S5. Characteristics for asymptomatic *C9ORF72* repeat expansion carriers and patients with c9ALS or c9ALS-FTD.

Variable	Asymptomatic *C9ORF72* repeat expansion carriers (N=27)	Patient with c9ALS or c9ALS-FTD (N=83)	P value
Age at CSF collection (Years)	46 (28, 32, 57, 63)	59 (36, 54, 64, 76)	<0.001
Gender (Male)	8 (29.6%)	51 (61.5%)	0.007
Time from onset to CSF collection (Months)	N/A	24 (0, 12, 31, 132)	N/A

The sample median (minimum, 25th percentile, 75th percentile, maximum) is given for continuous variables. P values result from Fisher's exact test or a Wilcoxon rank sum test.

Table S6. Poly(GP) in CSF collected longitudinally from *C9ORF72* repeat expansion carriers.

Patient number	Months since first CSF collection	Diagnosis at collection	Poly(GP) (ng/ml)
149	0.0	ALS	0.84
	9.1	ALS	0.82
150	0.0	ALS	0.78
	10.6	ALS	0.38
151	0.0	ALS	2.95
	4.4	ALS	3.18
154	0.0	ALS	3.91
	4.6	ALS	4.03
	10.4	ALS	3.58
	15.2	ALS	3.32
	22.6	ALS	2.89
155	0.0	ALS	0.52
	13.3	ALS	0.03
157	0.0	ALS	1.48
	3.2	ALS	1.68
	9.2	ALS	1.10
	13.6	ALS	1.23
163	0.0	ALS	0.21
	12.9	ALS	0.25
165	0.0	ALS	0.27
	6.7	ALS	0.15
	12.2	ALS	0.15
167	0.0	ALS	3.81
	5.3	ALS	3.69
169	0.0	ALS	0.25
	6.0	ALS	0.22
170	0.0	ALS	1.79
	6.0	ALS	1.62
193	0.0	ALS	1.06
	5.5	ALS	1.25
	17.7	ALS	1.50
195	0.0	ALS	0.00
	5.5	ALS	0.00
	17.5	ALS	0.00
197	0.0	ALS	1.37
	6.9	ALS	1.75
198	0.0	ALS	0.21
	5.1	ALS	0.12
199	0.0	ALS	0.79
	4.6	ALS-FTD	0.96
201	0.0	ALS	0.09
	6.0	ALS	0.17
216	0.0	ALS	1.14
	18.3	ALS	0.56
189	0.0	ALS-FTD	0.58
	6.2	ALS-FTD	0.74
	18.4	ALS-FTD	0.84
191	0.0	ALS-FTD	2.50

	6.2	ALS-FTD	2.41
	18.2	ALS-FTD	2.79
192	0.0	ALS-FTD	1.83
	6.2	ALS-FTD	1.35
194	0.0	ALS-FTD	0.56
	6.7	ALS-FTD	0.51
202	0.0	ALS-FTD	1.49
	5.5	ALS-FTD	1.77
223	0.0	ALS-FTD	0.83
	17.0	ALS-FTD	0.98
123	0.0	asymptomatic	0.13
	16.7	asymptomatic	0.03
124	0.0	asymptomatic	0.95
	15.6	asymptomatic	0.91
125	0.0	asymptomatic	0.29
	18.6	asymptomatic	0.18
126	0.0	asymptomatic	0.05
	16.7	asymptomatic	0.01
133	0.0	asymptomatic	0.51
	11.2	asymptomatic	0.56
136	0.0	asymptomatic	3.98
	12.9	asymptomatic	3.85
142	0.0	asymptomatic	1.09
	5.3	asymptomatic	1.10
	10.8	asymptomatic	1.29
	16.8	asymptomatic	1.87
143	0.0	asymptomatic	0.28
	6.2	asymptomatic	0.35
	19.1	asymptomatic	0.39
144	0.0	asymptomatic	0.55
	6.0	asymptomatic	0.65

Table S7. Secondary comparisons of CSF poly(GP) in patients with c9ALS or c9ALS-FTD.

Variable	N	Median (minimum, Q1, Q3, maximum) poly(GP)	P value	
			Unadjusted	Adjusted
Age at CSF collection			0.93	0.86[a]
< 59 years*	41	0.9 (0.0, 0.5, 1.7, 5.2)		
≥ 59 years*	42	0.8 (0.0, 0.3, 1.5, 3.9)		
Disease onset to CSF collection			0.91	0.61[a]
< 24 months*	41	0.9 (0.0, 0.5, 1.7, 5.2)		
≥ 24 months*	42	0.8 (0.0, 0.4, 1.5, 5.2)		
Gender			0.004	N/A[b]
Male	51	0.9 (0.0, 0.6, 2.2, 5.2)		
Female	32	0.8 (0.0, 0.2, 1.1, 3.3)		
Age of onset			0.89	0.98[a]
< 56.4 years*	41	0.8 (0.0, 0.5, 1.7, 5.2)		
≥ 56.4 years*	42	0.8 (0.0, 0.3, 1.5, 3.9)		
Onset site			0.55	0.60[a]
Limb	51	0.8 (0.0, 0.5, 1.5, 5.2)		
Bulbar	25	0.8 (0.0, 0.3, 1.6, 5.2)		
Other	4	1.3 (0.6, 0.6, 2.2, 2.5)		
Disease group			0.97	0.41[a]
ALS	71	0.8 (0.0, 0.5, 1.5, 5.2)		
ALS-FTD	12	0.8 (0.0, 0.6, 1.8, 2.5)		
ALSFRS-R score			0.99	0.98[a]
< 36*	29	0.8 (0.0, 0.5, 2.2, 5.2)		
≥ 36*	31	0.9 (0.0, 0.5, 1.5, 3.9)		
Behavioral impairment			0.10	0.58[c]
No behavioral impairment	16	0.5 (0.0, 023, 1.1, 5.2)		
Behavioral impairment	13	0.8 (0.3, 0.6, 1.8, 3.8)		
Cognitive impairment			0.018	0.12[c]
No cognitive impairment	15	0.5 (0.0, 0.2, 0.9, 1.8)		
Cognitive impairment	15	1.5 (0.0, 0.6, 2.5, 5.2)		

[a]Linear regression model adjusted for gender. [b]Gender was the only variable that was associated with poly(GP) in unadjusted analysis in c9ALS and c9ALS-FTD patients, and therefore no adjustment for other variables was made regarding this association. [c]Linear regression model adjusted for age at CSF collection, gender, and years of education. *Continuous variables were dichotomized using the median for purposes of presentation only; these were analyzed as continuous variables in the linear regression analysis.

Table S8. Neuropsychological test battery for Mayo Clinic and NIH.

Domain	Mayo Clinic	NIH
General Cognitive Screen	ALS Cognitive Behavioral Screen–Cognitive Form Clinical Dementia Rating Scale with Supplemental Items	Mattis Dementia Rating Scale-2
Executive Function	ALS Cognitive Behavioral Screen–Cognitive Form	D-KEFS Trails Number-Letter Switching D-KEFS Sorting D-KEFS 20 Questions Frontal Assessment Battery
Memory	Wechsler Memory Scale-Revised Digit Span California Verbal Learning Test-2 Short Form	Mattis Dementia Rating Scale-2 Memory Subscore
Visuospatial	Judgment of Line Orientation	D-KEFS Trails Visual Scanning Mattis Dementia Rating Scale-2 Construction
Language	Boston Naming Test Short Form Oral Phonemic and Semantic Fluency Written Phonemic and Semantic Fluency Five Word Spelling Test Written 4-Letter C Word Fluency	NACC FTLD Language Module D-KEFS Verbal Fluency
Emotion and Behavior	Beck Depression Inventory Neuropsychiatric Inventory–Questionnaire ALS Cognitive Behavioral Screen–Behavioral Form	ALS Depression Index Frontal Behavioral Inventory SF-8 Health Survey
Quality of Life	Neurology Quality of Life Scale	
Instrumental Activities of Daily Living	Modified Functional Assessment Questionnaire	

ALS: amyotrophic lateral sclerosis; D-KEFS: Delis Kaplan Executive Function System; FTLD: frontotemporal lobar degeneration; NACC: National Alzheimer's Coordinating Center

Table S9. Characteristics according to behavioral impairment in patients with c9ALS or c9ALS-FTD.

Variable	No behavioral impairment (N=16)	Behavioral impairment (N=13)	P value
Age at CSF collection (Years)	56 (36, 51, 61, 67)	61 (43, 56, 64, 71)	0.11
Time from onset to CSF collection (Months)	26 (6, 17, 40, 89)	26 (6, 19, 37, 82)	0.91
Gender (Male)	8 (50%)	13 (100%)	0.003
Education (Years)	14 (12, 12, 16, 18)	16 (12, 16, 18, 20)	0.017

The sample median (minimum, 25th percentile, 75th percentile, maximum) is given for continuous variables. P values result from Fisher's exact test or a Wilcoxon rank sum test.

Table S10. Characteristics according to cognitive impairment in patients with c9ALS or c9ALS-FTD.

Variable	No cognitive impairment (N=15)	Cognitive impairment (N=15)	P value
Age at CSF collection (Years)	56 (36, 50, 63, 67)	60 (43, 55, 63, 71)	0.48
Time from onset to CSF collection (Months)	25 (6, 16, 33, 89)	31 (6, 19, 41, 83)	0.41
Gender (Male)	8 (53.3%)	14 (93.3%)	0.035
Education (Years)	16 (12, 13, 16, 18)	16 (12, 13, 18, 20)	0.59

The sample median (minimum, 25th percentile, 75th percentile, maximum) is given for continuous variables. P values result from Fisher's exact test or a Wilcoxon rank sum test.

Table S11. Subject characteristics for PBMCs.

Sample number	*C9ORF72* repeat expansion	Diagnosis	Site of onset
PBMC1	N	Healthy	-
PBMC2	N	Healthy	-
PBMC3	N	ALS	Limb
PBMC4	N	ALS	Limb
PBMC5	N	ALS	Bulbar
PBMC6	N	ALS	Limb
PBMC7	N	ALS	Limb
PBMC8	N	ALS	Limb
PBMC9	N	ALS	Limb
PBMC10	N	ALS	Limb
PBMC11	N	ALS	Bulbar
PBMC12	N	ALS	Limb
PBMC13	N	ALS	n/a
PBMC14	N	ALS	Limb
PBMC15	N	ALS	Bulbar
PBMC16	N	ALS	Limb
PBMC17	N	ALS	Limb
PBMC18	N	ALS	Bulbar
PBMC19	N	ALS	Bulbar
PBMC20	N	ALS	Limb
PBMC21	N	ALS	Limb
PBMC22	N	ALS	Bulbar
PBMC23	N	ALS	n/a
PBMC24	N	ALS	Bulbar
PBMC25	N	ALS	Limb
PBMC26	N	ALS	n/a
PBMC27	N	ALS	n/a
PBMC28	N	ALS	Limb
PBMC29	N	ALS	Limb
PBMC30	N	ALS	Limb
PBMC31	N	ALS	Limb
PBMC32	N	ALS	Limb
PBMC33	N	ALS	Limb
PBMC34	N	Psychiatric disorder	-
PBMC35	Y	Asymptomatic	-
PBMC36	Y	Asymptomatic	-
PBMC37	Y	Asymptomatic	-
PBMC38	Y	Asymptomatic	-
PBMC39	Y	Asymptomatic	-
PBMC40	Y	Asymptomatic	-
PBMC41	Y	Asymptomatic	-
PBMC42	Y	Asymptomatic	-
PBMC43	Y	Asymptomatic	-
PBMC44	Y	Asymptomatic	-
PBMC45	Y	Asymptomatic	-
PBMC46	Y	Asymptomatic	-
PBMC47	Y	Asymptomatic	-
PBMC48	Y	Asymptomatic	-
PBMC49	Y	Asymptomatic	-
PBMC50	Y	ALS	Limb
PBMC51	Y	ALS	Limb

PBMC52	Y	ALS	Limb
PBMC53	Y	ALS	Limb
PBMC54	Y	ALS	Limb
PBMC55	Y	ALS	Limb
PBMC56	Y	ALS	Bulbar
PBMC57	Y	ALS	Limb
PBMC58	Y	ALS	Bulbar and limb
PBMC59	Y	ALS	Limb
PBMC60	Y	ALS	Limb
PBMC61	Y	ALS	Limb
PBMC62	Y	ALS	Bulbar
PBMC63	Y	ALS	Bulbar
PBMC64	Y	ALS	Limb
PBMC65	Y	ALS	Limb
PBMC66	Y	ALS	Bulbar
PBMC67	Y	ALS	Limb
PBMC68	Y	ALS	Bulbar
PBMC69	Y	ALS	Bulbar
PBMC70	Y	Psychiatric disorder	-

n/a, data not available.

Table S12. Subject characteristics for lymphoblastoid cell lines.

Line number	*C9ORF72* repeat expansion	Diagnosis	Site of onset
LCL1	N	Healthy	-
LCL2	N	Healthy	-
LCL3	N	ALS	Bulbar
LCL4	N	ALS	n/a
LCL5	N	ALS	n/a
LCL6	N	ALS	Limb
LCL7	N	ALS	n/a
LCL8	Y	Asymptomatic	-
LCL9	Y	Asymptomatic	-
LCL10	Y	Asymptomatic	-
LCL11	Y	Asymptomatic	-
LCL12	Y	Asymptomatic	-
LCL13	Y	Asymptomatic	-
LCL14*	Y	Asymptomatic	-
LCL15	Y	ALS	Limb
LCL16*	Y	ALS	Limb
LCL17	Y	ALS	Bulbar
LCL18	Y	ALS	Limb
LCL19	Y	FTD	-

*Lines used for c9ASO treatment studies. n/a, data not available.

Table S13. Subject characteristics for iPSC lines.

Line number	*C9ORF72* repeat expansion	Gender	Diagnosis	Site of onset	Age at onset	Estimated G_4C_2 repeat units*
JH082	N	M	Healthy	-	-	<30
JH018	N	F	Healthy	-	-	<30
CS25	N	M	Healthy	-	-	<30
JH033	Y	M	ALS	Limb	62	900
JH034	Y	F	ALS	Bulbar	63	**500**, 400
JH078	Y	M	ALS	Bulbar	58	500
JH092	Y	M	ALS	Bulbar	49	700
ALS50	Y	M	ALS	Limb	54	700
ALS75	Y	M	ALS	Limb	49	**600**, 900
CS30	Y	F	ALS	Bulbar	51	70

*Measured by Southern blot. If two bands were detected, the number of repeats estimated from the dominant band is in bold.